species can grow in air enriched with 10% CO_2. There is no growth below pH 4.5 or above 8.5. Chemoorganotrophs, actively ferment carbohydrates, with the production mainly of acetic and lactic acids in the molar ratio of 3:2; CO_2 is not produced. Butyric and propionic acids are not produced. Catalase negative (rarely positive when grown in air with added CO_2). Usually require various vitamins. The optimum growth temperature is 37–41°C. Found in the mouth and intestinal tract of warm-blooded vertebrates, in insects, and in sewage; have been implicated in human infective processes but usually are considered nonpathogenic.

Type species: *Bifidobacterium bifidum.*

Differentiation of the species of the genus **Bifidobacterium:** This differentiation requires specialized techniques for strict anaerobiosis and metabolic studies.

Genus Brachybacterium

Editorial note: The genus *Brachybacterium* was not included in *Bergey's Manual of Systematic Bacteriology.* It was established by Collins et al. (Int. J. Syst. Bacteriol. 38: 45–48, 1988) for the new species *B. faecium,* which is similar to *Brevibacterium* but with a distinctive pattern of lipids and menaquinones and the ability to acidify glucose in peptone media.

In young cultures, slender rods, 0.5–0.75 × 1.5–2.5 μm, are irregular in shape. Some cells are arranged in V formation, and these segment into cocci in old, stationary-phase cultures. Gram positive, nonmotile, nonsporing, not acid-fast. Aerobic, colonies white or pale yellow. Chemoorganotrophic, metabolism respiratory, yielding acid from glucose and some other carbohydrates. Catalase positive, oxidase negative, and may reduce nitrate. The optimum temperature is 25–30°C. Isolated from poultry litter.

Type (and only) species: *Brachybacterium faecium.*

Characteristics of the species: As described for the genus.

Genus Brevibacterium

Cells in young cultures are irregular rods, 0.6–1.2 × 1.5–6 μm, arranged singly or in pairs and often at an angle to give V formations. Branching may occur, but a mycelium is not formed. In older cultures the rods segment into small cocci. Gram positive but easily decolorized. Nonmotile, nonsporing, not acid-fast. Strict aerobes; colonies may show yellow-orange or purple pigmentation. Chemoorganotrophs, metabolism respiratory. Little or no acid is produced from glucose or other carbohydrates. Catalase positive, gelatin and casein are usually hydrolyzed, often produce methanethiol from L-methionine. The optimum growth temperature is 20–35°C. Widely distributed in dairy products and are found on human skin.

Type species: *Brevibacterium linens.*

Differentiation of the species of the genus **Brevibacterium:** See Table 20.6.

Editorial note: Four definite species of the genus were included in *Bergey's Manual of Systematic Bacteriology,* and these are shown in Table 20.6. Additional species that have been poorly studied or are of uncertain generic position were also treated, and some are now included under the genera *Arthrobacter, Corynebacterium,* and *Microbacterium.*

Genus Butyrivibrio

Editorial note: The genus *Butyrivibrio* has been regarded as Gram positive or Gram negative, and for this reason it is noted in Group 20 as well as in Group 6.

Curved rods, 0.3–0.8 × 1.0–5.0 μm, are arranged singly or in chains or filaments, which may be helical. Stain Gram negative, but the cell wall is of the Gram-positive type. Cells are motile by a few polar or subpolar flagella; motility is rapid and vibratory, though often only a few cells in a culture are motile. Strictly anaerobic. Growth is slow below 30°C; there is no growth at 50°C (optimum 37°C). Chemoorganotrophic metabolism fermentative; glucose is fermented with butyrate as a major product and sometimes lactate. There is little growth in the absence of carbohydrates, but cellulose, starch, and other polysaccharides are often attacked. Catalase negative, may reduce nitrate. Occur in the rumen of ruminants and occasionally in mammalian feces; they are nonpathogenic.

Type species: *Butyrivibrio fibrisolvens.*

Differentiation of the species of the genus **Butyrivibrio:** See Table 20.7.

Cells are chemoorganotrophic. Acetate is the only major utilizable carbon source, and cells are catalase positive. The optimum growth temperature is 25–30°C. These bacteria are associated with cattle dung.

Type species: *Caryophanon latum.*

Differentiation of the species of the genus Caryophanon: See Table 19.4.

Genus Erysipelothrix

Straight or slightly curved, slender rods, 0.2–0.4 × 0.8–2.5 μm, have a tendency to form long filaments, often 60 μm or more long. Gram-positive, nonmotile, nonsporing cells are without capsules. They are not acid-fast and are chemoorganotrophic. Cells are facultatively anaerobic and catalase negative. The optimum temperature is 30–37°C. Fermentative activity is weak, with acid but no gas from glucose and a few other carbohydrates. *Erysipelothrix* species are widely distributed in nature and are usually parasitic on mammals, birds, and fish; some strains are pathogenic for mammals and birds.

Type species: *Erysipelothrix rhusiopathiae.*

Differentiation of the species of the genus Erysipelothrix: A second species of the genus, *Erysipelothrix tonsillarum*, was described by Takahashi et al. (Int. J. Syst. Bacteriol. *37*: 166–168, 1987); this species can be differentiated from *E. rhusiopathiae* only by DNA-DNA homology. However, the strains belong to one serovar, serovar 7, and show little evidence of virulence for swine.

Genus Kurthia

Regular, unbranched rods with rounded ends in young cultures, 0.8–1.2 × 2–4 μm, occur in long chains. Older cultures (over 2 days) are usually composed of coccoid cells. Gram-positive cells are usually motile by peritrichous flagella. They do not form endospores and are not acid-fast. Cells are strictly aerobic. Growth on yeast nutrient agar shows rhizoid colonies, with loops and whorls of chains of rods at the edge ("Medusa-head appearance"), and on nutrient gelatin slants the growth has a "bird's feather" appearance. Cells are chemoorganotrophs, with a respiratory, not fermentative, metabolism, with weak acidity from glucose. The optimum growth temperature is 25–30°C. Nonpathogenic, *Kurthia* species are widely distributed in the environment and are common in animal feces and meat products.

Type species: *Kurthia zopfii.*

Differentiation of the species of the genus Kurthia: See Table 19.5.

Genus Lactobacillus

Cells are rod-shaped and usually regular, 0.5–1.2 × 1.0–10.0 μm. They are usually long rods but sometimes are almost coccoid, commonly in short chains. Gram-positive, nonsporing cells are rarely motile by peritrichous flagella. Facultative anaerobes, sometimes microaerophilic, grow poorly in air but better under reduced oxygen tension; some are anaerobes on isolation. Growth is generally enhanced by 5% CO_2. Colonies on agar media are usually 2–5 mm, convex, entire, opaque, and without pigment. Chemoorganotrophs, these cells require rich, complex media; their metabolism is fermentative and saccharoclastic; at least half of the end-product carbon is lactate. Nitrates are not reduced, gelatin is not liquefied, and cells are catalase and cytochrome negative. The major C_{18} straight-chain fatty acid is *cis*-vaccenic. The optimum growth temperature is 30–40°C. Lactobacilli are widely distributed in the environment, especially in animal and vegetable food products; they normally inhabit the gastrointestinal tract of birds and mammals and the mammalian vagina. They are rarely pathogenic.

Type species: *Lactobacillus delbrueckii.*

Differentiation of the species of the genus Lactobacillus: This large genus requires special expertise to identify the species; many test reactions are weak and dependent on the composition of the media and the exact cultural conditions.

Genus Listeria

Regular, short rods, 0.4–0.5 × 0.5–2 μm with rounded ends, are sometimes almost coccoid, occurring singly or in short chains and less often in long filaments. Cells are Gram positive, nonsporing, not acid-fast, and not encapsulated. They are motile by a few

white, and opaque, with an undulant edge; **no aerial filaments.** Chemoorganotrophic, requiring nutritionally rich media; fermentative, yielding acid but no gas from many carbohydrates. **The major end products** of glucose fermentation are **acetic and propionic acids. Catalase negative,** indole negative, reduce nitrate to nitrite. The optimum growth temperature is 35–37°C. Inhabit the human oral cavity and are occasionally involved in infective processes.

Type (and only) species: *Arachnia propionica.*

Characteristics of the species: As described for the genus.

Genus Arcanobacterium

Slender, irregular rods, 0.3–0.8 × 1.0–5.0 μm, in young cultures; cells may show clubbed ends **sometimes arranged in V formation but there are no filaments.** In older cultures, organisms **segment into short, irregular rods and cocci. Gram positive, nonmotile,** not acid-fast, without endospores. **Facultatively anaerobic.** Grow slowly on nutrient agar; growth is better on horse blood agar, giving small, convex, translucent colonies surrounded by a **zone of complete hemolysis** after 2 days at 37°C. Growth is enhanced by the addition of CO_2. Chemoorganotrophic, requiring nutritionally rich media. **Metabolism is fermentative,** yielding acid but no gas from glucose and a few other carbohydrates, with the production of mainly acetic, lactic, and succinic acids. Usually **catalase negative;** some strains show weak activity. Indole negative, nitrate is reduced to nitrite. The optimum growth temperature is 37°C. Obligate parasites of the pharynx of humans and farm animals; occasionally, they cause pharyngeal or skin lesions.

Type (and only) species: *Arcanobacterium haemolyticum.*

Characteristics of the species: As described for the genus.

Genus Arthrobacter

Cells in young cultures are irregular rods, 0.8–1.2 × 1.0–8.0 μm, **often V-shaped** and with clubbed ends, **but there are no filaments. As growth proceeds the rods segment into small cocci,** 0.6–1.0 μm in diameter, **arranged singly, in pairs, and in irregular clumps. This marked rod-coccus growth cycle** is characteristic of *Arthrobacter* and *Pimelobacter;* stationary phase cultures consist almost entirely of cocci. **Gram positive** but easily decolorized. The rods of some species are motile. Nonsporing, not acid-fast. **Aerobic.** Chemoorganotrophic, usually grow on simple media plus biotin, with an **oxidative metabolism.** Little or no acid and no gas is produced from glucose and other carbohydrates. **Catalase positive.** The optimum growth temperature is 25–30°C. Widely distributed in the environment, principally in soils.

Type species: *Arthrobacter globiformis.*

Differentiation of the species of the genus **Arthrobacter:** The differentiation of species is difficult because many are still poorly studied and comparative data are scanty.

Genus Aureobacterium

Irregular short rods, 0.4–0.6 × 0.6–3 μm, occur singly or in irregular groups; **many cells are arranged in V forms.** In older cultures rods become shorter but a **marked rod-coccus cycle does not occur.** Branching is uncommon; **no mycelium is produced.** Cells are **Gram positive** in young cultures. Some species are motile. Nonsporing, not acid-fast. **Aerobic. Colonies are pigmented in shades of yellow,** and the pigment is nondiffusing. Chemoorganotrophic, require nutritionally rich media. Metabolism is respiratory, yielding weak acid but no gas from glucose and other carbohydrates. The optimum temperature is 25–30°C. **Catalase positive.** Some species require siderophores such as terregens factor (found in soil extract). Found in soil and dairy products but are probably widely distributed in the environment.

Type species: *Aureobacterium liquefaciens.*

Differentiation of the species of the genus **Aureobacterium:** See Table 20.5.

Genus Bifidobacterium

Rods of very varied shapes, 0.5–1.3 × 1.5–8 μm, usually **somewhat curved and clubbed** and are often **branched.** Arranged singly, in pairs, **in V arrangements,** sometimes in chains, in palisades of parallel cells, or in rosettes. Occasionally exhibit swollen coccoid forms. **Gram positive, often stain irregularly. Nonmotile,** nonsporing, **non-acid-fast. Anaerobic.** A few

that *P.* ...es play a role in acne vulgaris. The mechan... of antibiotic resistance has not yet been determined but does not appear to reflect the acquisition of genes from other organisms. Nevertheless, erythromycin resistance is phenotypically indistinguishable from that of the majority of bacteria where resistance is due to methylation of the 23S ribosomal RNA since most resistant *P. acnes* are inducibly or constitutively resistant to macrolide, lincosamide and streptogramin B antibiotics (Eady et al. 1989a, 1989b).

Prior to topical therapy, only a few per cent of strains were reported resistant to erythromycin or tetracycline though most were resistant to aminoglycosides and fusidic acid (Höffler, Niederau and Pulverer 1980); all were sensitive to penicillins and cephalosporins.

4 PROPIONIBACTERIUM GRANULOSUM

P. granulosum is also found in the sebaceous glands and on the human skin surface but generally at densities about 100-fold lower than those of *P. acnes*. Colonies on lipid-containing media are larger than those of *P. acnes* after incubation for 3 days anaerobically and are generally reddish pigmented. *P. granulosum* is not susceptible to *P. acnes* phage, is DNAase-positive, gelatinase- and casein hydrolase-negative, indole- and nitrate-negative. The DNA of *P. granulosum* has only about 12–15% homology with that of *P. acnes* or *P. avidum*.

Like *P. acnes*, *P. granulosum* may be found in some lesions of the skin and deeper tissue and is occasionally recognized as an opportunist pathogen such as in septicaemia of an immunocompromised host (Branger, Bruneau and Goullet 1987).

Studies of *P. granulosum* or *P. avidum* as immunostimulators (Höf and Pulverer 1987) are reminiscent of the use of '*Corynebacterium parvum*', a commercially available product now acknowledged to be a mixture of the *Propionibacterium* spp. found on human skin (Cummins and Johnson 1974). The effects of '*C. parvum*' were varied and depended on the experimental conditions (Miles and Scott, 1978, Hart 1985); this is no doubt a reflection of the effect of propionibacterium cell wall on the complement system.

5 PROPIONIBACTERIUM AVIDUM

P. avidum is frequently found on the moist areas of skin, such as the axillae, and is very much less common on lipid-rich areas (Nordstrom and Noble 1984) but, like the other species, is most common on post-pubertal individuals. After anaerobic incubation for 3 days, colonies are larger than those of *P. acnes* and are generally not pigmented. *P. avidum* is not susceptible to *P. acnes* phage, is DNAase-positive, gelatinase- and casein hydrolase-positive, indole- and nitrate-negative. *P. avidum* is less fastidious in amino acid requirements than the other 2 species (Ferguson and Cummins 1978), but shows about 50% homology of 16S RNA sequences with *P. acnes* (Charfreitag and Stackebrandt 1989).

As with the other skin species, *P. avidum* is occasionally reported from deep lesions such as splenic abscess (Dunne et al. 1986).

6 PROPIONIBACTERIUM PROPIONICUS

P. propionicus was formerly assigned to the genus *Actinomyces*, chiefly because branched bacilli may be seen. It was then transferred to the genus *Arachnia* and from there to *Propionibacterium* (Charfreitag, Collins and Stackebrandt 1988) on the basis of sequence homology of ribosomal RNA. On grammatical grounds the specific epithet should read 'propionicum'.

Anaerobic growth after 48 h on blood agar media is grey-white, dry and rough. The crumb-like colonies cause pitting of the agar. Microscopically cells may be filamentous or branching. Much propionic acid is produced. Porphyrin production is sufficient to produce red fluorescence of the colonies under UV light.

P. propionicus is a normal inhabitant of the human oral cavity but is found causing infection of the lachrymal apparatus, especially in older women (Brazier and Hall 1993, Caukas et al. 1993). Although some eye infections with *P. acnes* are reported, it may be that these are in fact *P. propionicus*. Eye infections may be difficult to treat with topical antibiotics and strains are reported resistant to gentamicin, neomycin and sulphonamides (Seal et al. 1981).

7 PROPIONITRAS INNOCUUM

P. innocuum was originally described on the basis of its cell wall composition as a new coryneform from human skin (Pitcher 1976). It was formerly allocated to the genus *Propionibacterium* (Pitcher and Collins 1991) from which it differs chiefly in growing well aerobically and in possessing arabinose in its cell wall. It has now been elevated to monotypic genus status as *Propioniferax innocuum* (Yokata et al. 1994).

BIFIDOBACTERIUM

GENUS DESCRIPTION

Bifidobacterium spp. are pleomorphic gram-positive rods showing true and false branching. They are non-motile and non-sporing. Most species are strictly anaerobic; they grow between 20 and 45°C with optimum growth at 38°C. The organisms are aciduric, but killed by heat at 60°C in 5 min. They ferment various carbohydrates with production of acetic and lactic acids in the ratio 3:2. CO_2 is not produced. Glucose metabolism is characteristically and exclusively by the fructose-6-phosphate shunt. There is no proteolytic activity. They do not form oxidase, indole or H_2S and are generally non-pathogenic to man and animals. *Bifidobacterium* spp. are normal flora of the mouth and intestine. The G + C content of the DNA is c. 60 mol%. The type species is *Bifidobacterium bifidum*.

9 INTRODUCTION AND HISTORICAL PERSPECTIVE

The first member of this genus to be recognized was isolated from infants' stools by Tissier (1900), who

called it *Bacillus bifidus*. The classification of the bifidobacteria has presented difficulties, but most workers consider that the bifidobacteria should be classed in a genus of their own, as suggested by Orla-Jensen (1924). This has been confirmed by phylogenetic studies that have demonstrated that the bifidobacteria are confined to a single deep cluster within the high G + C gram-positive group (Maidak et al. 1994). Furthermore, all the bifidobacteria share the property peculiar to them among the non-sporing gram-positive anerobes of degrading glucose by the fructose-6-phosphate shunt. Species definitions have been the subject to extensive revision. Several biotypes or species were described by Dehnert (1957, 1961). Gyllenberg and Carlberg (1958) and Reuter (1963, 1971) recognized and named 8 separate species from human sources. However, by means of DNA homology studies, Scardovi and his colleagues (1971) found that some of these species were homologous, and they reduced the number to 5, whilst adding 4 more. Subsequent studies have proposed a number of new species; they are currently 29, 10 of which have been isolated from humans. Bifidobacteria make up a high proportion of the bacteria in the gut flora of human infants and adults.The protective effects of bifidobacteria against enteric infection and cancer are now widely appreciated and the inclusion of bifidobacteria in probiotic products is widespread.

12 METABOLISM

Biochemically, one of the more striking features of bifidobacteria is the formation of acetic acid in addition to lactic acid during the fermentation of glucose. This property, and the failure to form detectable gas, separate the bifidobacteria from the heterofermentative group of lactobacilli (Beerens, Gerard and Guillaume 1957). The absence of propionic acid from the products of fermentation separates *Bifidobacterium* from *Propionibacterium*. Glucose is utilized by the fructose-6-phosphate shunt, and detection of fructose-6-phosphate phosphoketolase is the most reliable test for assigning an organism to this genus (for method, see Scardovi 1986). Urease production is uncommon among human isolates, but ureolytic strains occur in all species. One of the animal species, *Bifidobacterium suis*, is most often strongly ureolytic. The catalase reaction is usually negative, but some oxygen-tolerant strains liberate O_2 from H_2O_2 when grown in air + 10% CO_2. Nitrate reduction cannot be demonstrated by the usual tests. Many of the bifidobacteria isolated from the human gut are able to hydrolyse cholic acid and conjugated bile acids such as sodium glycocholate and sodium taurocholate (Drasar and Hill 1974, Ferrari, Pacini and Canzi 1980).

13 ECOLOGY

Bifidobacteria are found in the human mouth, the lower gut of humans and animals and in sewage; they are occasionally isolated from clinical material.

14 MORPHOLOGY

A wide variety of cell morphologies are displayed and even a single strain may appear different under different cultural conditions. The classic bifid club-shaped protusions are shown by most species under conditions of nutrient limitation. Otherwise, morphologies range from the large, curved, irregular-shaped cells of *B. bifidum* and the palisade arrangement of *Bifidobacterium angulatum* to the characteristic star-like clusters of *Bifidobacterium asteroides*. Although it has been suggested that species identification is possible by examination of cell morphology (Scardovi 1986), this is not recommended without substantial experience of the genus.

15 GROWTH CHARACTERISTICS

Most species are strict anaerobes, but a few species, isolated from animals or bees, will tolerate O_2 in the presence of added CO_2, growing in 90% air + 10% CO_2.

16 CELL WALL CHEMISTRY AND TAXONOMY

Studies of cell wall murein of bifidobacteria have shown that the amino acid composition of the peptide side chains can be useful in species identification. However, closely related species may have the same cross-links, e.g. *Bifidobacterium longum* and *Bifidobacterium infantis* (Kandler and Lauer 1974). Other chemotaxonomic studies, such as the analysis of cellular fatty acids and phosphoglycerides, have not proved useful for species identification (Exterkate et al. 1971, Veerkamp 1971).

The antigenic structure is complex and most workers have found a fairly high degree of strain specificity by agglutination reactions. The antigens taking part in these reactions are heat stable; the results are the same whether living or boiled organisms are used for the preparation of antisera. Unlike the lactobacilli, bifidobacteria do not seem to contain an extractable precipitinogen.

17 SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

Bifidobacteria are uniformly susceptible to benzylpenicillin, the macrolides and lincosamines, chloramphenicol and vancomycin, but are usually resistant to the aminoglycosides, nalidixic acid and metronidazole.

16 CLASSIFICATION

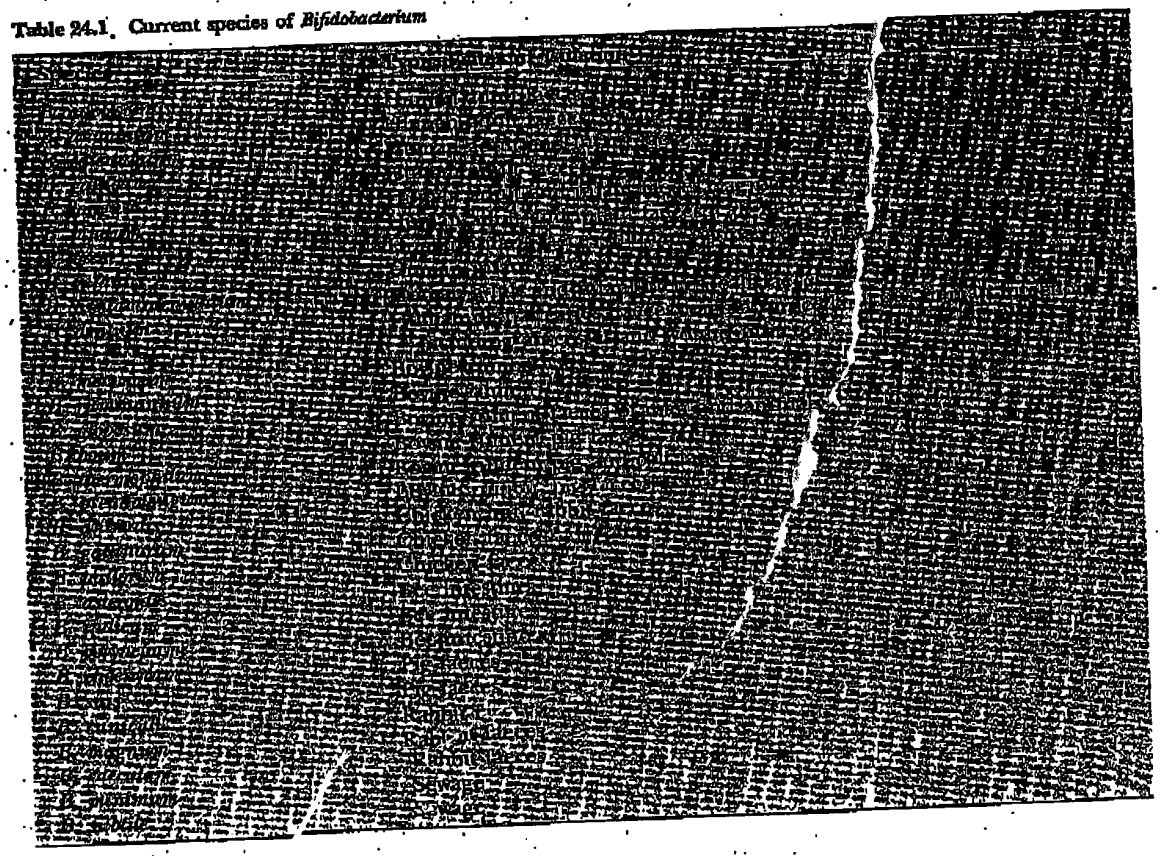
Study of the DNA composition reveals a G+C content of 57.2–64.2 mol%, with a mean of 60.1. These figures differ from the much lower ones of 38.0–52.5 for the lactobacilli and the higher ones of 66.4–70.4 for the propionibacteria (Sebald, Gasser and Werner 1965, Werner, Gasser and Sebald 1965). Substantial revision of the classification has taken place in recent years, based primarily on DNA–DNA homology data. *B. bifidum* and *Bifidobacterium adolescentis* have been shown to form distinct genetic groups. *B. bifidum* biotypes a and b are closely related to each other, as are the 4 biotypes of *B. adolescentis*. The 4 species, *Bifidobacterium dentium*, *Bifidobacterium angulatum*, *Bifidobacterium catenulatum* and *Bifidobacterium pseudocatenulatum*, which are difficult to distinguish from *B. adolescentis* on the basis of fermentation patterns, are genetically distinct from each other and from *B. adolescentis*. *Bifidobacterium longum* and *Bifidobacterium infantis* are the most closely related species with about 50% homology, and *Bifidobacterium breve* shows 40–50% homology with *B. infantis* (Scardovi et al. 1971, Biavati et al. 1984).

At present, 29 species in all are recognized (Table 24.1). Of these, 10 (*B. bifidum*, *B. breve*, *B. gallicum*, *B. infantis*, *B. longum*, *B. adolescentis*, *B. catenulatum*, *B. dentium*, *B. angulatum* and *B. pseudocatenulatum*) have been isolated from the human mouth, faeces or vagina; 14 other species have been found in the intestines and faeces of other vertebrate animals, including pigs, cattle, chickens and rabbits. Three species are unique to the intestine of the honey bee and 2 species have been found only in sewage. Species have been proposed on the basis of DNA–DNA homology studies supported by phenotypic tests and immunological and chemotaxonomic investigations.

17 LABORATORY ISOLATION AND IDENTIFICATION

The nutritional requirements of bifidobacteria are still imperfectly known. Since the discovery that milk stimulated the growth of *B. bifidum*, considerable attention has been given to the study of 'bifidus factors' in human milk, but these studies have added little to our knowledge (Poupard, Hussain and Norris 1978). Bifidobacteria are very heterogeneous in their requirements for growth factors and vitamins; riboflavin and pantothenate are the most common requirements, but nicotinic acid, pyridoxine, thiamine,

Table 24.1 Current species of *Bifidobacterium*



folic acid and *p*-aminobenzoic acid may also be needed (Scardovi 1986). Most species are able to use ammonium salts as the sole source of nitrogen, but some species isolated from animals will not grow in the absence of organic nitrogen.

Many different media have been devised for isolating or enumerating the bifidobacteria in faeces and sewage. The media contain a carbohydrate such as glucose, lactose, fructose or maltose and complex mixtures of peptones, yeast extract, growth factors and reducing agents. Antibiotics, particularly aminoglycosides, have been used to improve selectivity, but no universally satisfactory selective medium is at present available. Non-selective media that give good growth of the strains present in the habitat being studied are to be preferred (see Scardovi 1981).

Fermentation tests have been used traditionally to distinguish individual species. However, some species such as *B. infantis*, *B. longum*, *B. dentium* and *B. adolescentis* show similar patterns of sugar fermentation and this method cannot be used reliably for identification purposes. Biavati, Scardovi and Moore (1982) showed that the generation protein profiles of soluble wholesale proteins, using SDS-PAGE, was a reliable method for the identification of species. Members of a single species have identical or nearly identical protein patterns. This method has been widely used subsequently both for identification and as a method for the preliminary characterization of new strains for classification purposes. Recently developed molecular methods have also been employed for this purpose. Ribotyping, using 23S ribosomal RNA to probe digested chromosomal DNA, has resulted in both species and strain identification (Mangin et al. 1994). Oligonucleotide probes, designed from 16S rRNA sequence data, have been shown to be specific for *B. adolescentis*, *B. breve* and *B. longum*; probes for *B. bifidum* and *B. infantis* showed specificity adequate for the identification of isolates from human material (Yamamoto, Morotomi and Tanaka 1992). Extension and refinement of these approaches should permit rapid and even automated identification in the foreseeable future.

Although fermentation tests with simple sugars do not allow discrimination of species (Gavini et al. 1991), the use of complex carbohydrates has been shown by Crociani et al. (1994) to discriminate between species commonly isolated from human specimens (Table 24.2).

Isoenzyme patterns (of transaldolases and 6-phosphogluconate dehydrogenases) have proved useful for characterizing species of *Bifidobacterium*. Antisera against purified transaldolases have been used to distinguish groups of species from different habitats (Sgorbati and London 1982). Furthermore, β-galactosidase electrophoretic patterns successfully differentiated animal and human species of bifidobacteria and were more discriminatory than numerical analysis based on 45 tests in a comparative study (Roy, Berger and Reuter 1994).

18 Role in normal flora of humans

Bifidobacteria are reported to play an important regulatory role in the large intestine, controlling pH and protecting against infection by exogenous pathogens, particularly in infants (Modler, McKellar and Yaguchi 1990, Saavedra et al. 1994). It has further been proposed that bifidobacteria may offer protection against cancer, not only of the lower gut but also at other body sites (Reddy and Rivenson 1993). These findings have led to considerable interest in the use of bifidobacteria in probiotics and a number of dairy products, which include bifidobacteria, are being developed (Fuller 1989, Puhan 1990). The need for quality control and safety of such products has stimulated taxonomic studies of the genus and the development and refinement of identification systems.

The species most often found are: in babies, *B. infantis* and *B. breve*, and in adults, *B. adolescentis*, *B. longum* and *B. pseudocatenulatum* (Dehnert 1957, 1961, Reuter 1963, Beerens, Romond and Neut 1980, Biavati et al. 1984, Mitsuoka 1984). *B. dentium* is part of the normal oral flora and can be isolated readily from dental plaque (Biavati, Scardovi and Moore 1982).

19 Pathogenicity and virulence factors

B. dentium and 2 unnamed taxa are associated with dental caries and periodontal disease although whether they play a pathogenic role in these diseases is unknown (Moore et al. 1983). *B. dentium* has also been isolated from clinical specimens obtained from a variety of sites. These are principally mixed infections, typically abscesses or wound infections around the head and neck area or the lungs. *B. dentium* can probably be classed as an opportunist pathogen like many other members of the oral microflora. Other bifidobacteria that have been isolated from clinical material, albeit rarely, include *B. longum*, *B. adolescentis* and *B. breve*, recovered from abscesses, urinary tract infections and septicaemia (Darbas et al. 1991).

20 Bifidobacterium bifidum

B. bifidum is common in the faeces of breast-fed and bottle-fed infants and in the faeces of adults and of animals. It is non-pathogenic to man and laboratory animals. The G + C content of DNA is c. 60.1 mol% and DNA–DNA homology studies show *B. bifidum* to be a distinct species related to but separate from other bifidobacteria.

In faeces it is a delicate bacillus, about 4 μm long and 0.7 μm broad, with tapering, pointed ends. Arranged in pairs end to end, with the distal ends pointed and the proximal ends swollen, they generally lie parallel to one another, rarely intertwined. Two or 3 bacilli often radiate from a single point, forming a Y-shaped structure; clubbed forms and forms ending

ABIOTROPHIA

Members of the genus *Abiotrophia*, which contains 2 species, are the so-called nutritionally variant streptococci (NVS). They grow as satellite colonies around other microorganisms and in complex media only when supplemented with sulphydryl compounds such as cysteine. They have previously been referred to as satellite or symbiotic streptococci, thiol-requiring streptococci, vitamin B_6- or pyridoxal-dependent streptococci or NVS. They were previously considered nutritional variants of other streptococcal species, in particular *S. mitis*, but Bouvet and coworkers (1985, 1989) demonstrated that they form 2 distinct taxa proposed as '*Streptococcus adjacens*' and '*S. defectivus*' respectively. Subsequent comparisons of 16S rRNA sequences revealed that they are quite distinct from other species in the genus *Streptococcus*, which led to the proposal that they be placed in a new genus *Abiotrophia* as *A. adjacens* and *A. defectiva*, respectively (Kawamura et al. 1995b).

Members of the 2 species form minute α-haemolytic colonies on sheep blood agar supplemented with 10 mg l^{-1} pyridoxal hydrochloride or 100 mg l^{-1} cysteine. Their cell morphology depends upon growth conditions and phase. When cultivated in pyridoxal-or cysteine-supplemented complex media they are pleomorphic with chains that include cocci, coccobacilli, and rod-shaped cells. A tendency towards rod formation is observed in the stationary growth phase. In a semisynthetic medium (CDMT) they form small ovoid cocci (diameter 0.4–0.55 μm) which occur singly, in pairs or in chains of variable length. They do not produce extracellular polysaccharides from sucrose and may be easily distinguished from the mitis group of streptococci by their growth characteristics and the production of pyrrolidonyl-arylamidase (Bouvet, Grimont and Grimont 1989). A comprehensive study of their enzymatic activities have been presented by Beighton et al. 1995).

Abiotrophia species form part of the resident microflora of the human upper respiratory tract and may be isolated from vaginal and intestinal tracts. Like most members of the mitis group of streptococci they have been isolated from various human infections including subacute endocarditis, brain abscesses and wound infections, and from urine (Ruoff 1991).

LACTOBACILLUS

[redacted]

Lactobacilli are straight or curved rods of varying length and thickness, with parallel sides, arranged singly or in chains, sometimes filamentous or pleomorphic, without branching, clubbing or bifid formation. They are gram positive and non-sporing. Colonies on agar media are usually small. They have complex nutritional requirements. Growth is favoured by anaerobic or microaerophilic conditions and by carbon dioxide. Energy is obtained by the fermentation of sugars. Glucose is fermented, and either lactic acid alone or lactic acid along with other volatile acids and carbon dioxide is formed. Most strains have cell wall bound proteinases and peptidases. There is no production of catalase, oxidase or indole and no reduction of nitrate. The organisms are readily killed by heat but unusually tolerant of acid. Lactobacilli are widely distributed in fermenting vegetable and animal products and in the alimentary tract of humans and animals. They are rarely pathogenic for humans. The G+C content of DNA is 32–53 mol%. The genus includes 56 recognized species. The type species is *Lactobacillus delbrueckii*.

[redacted]

The type species of the genus *Lactobacillus*, *L. delbrueckii*, was originally isolated from milk by Leichmann (1896). A similar bacillus was observed by Döderlein in 1892 in the vaginal secretion of women, but the identity of this organism is in doubt (Sharpe 1981). In 1900 Moro cultured a slender gram-positive bacillus, *L. acidophilus*, from the faeces of breast-fed babies. Lactobacilli from cheese were named *L. casei* by Orla-Jensen (1904) and Heinemann and Hefferan (1909) isolated lactobacilli from human saliva, gastric juice, soil and various foods. An organism isolated from carious teeth and named *L. odontolyticus* (McIntosh et al. 1922, 1924) is probably the same as *L. plantarum* which was described by Pederson (1936). For other references on early work with lactobacilli see previous editions of this book.

The decision to describe *Streptococcus* and *Lactobacillus* in the same chapter follows Orla-Jensen's (1919, 1943) concept of a cluster of 'lactic-acid bacteria'. Orla Jensen's primary division was between the homofermentative thermobacteria and streptobacteria on one hand and the heterofermentative betabacteria on the other. The streptobacteria will grow at 15°C and most thermobacteria at 45°C. One organism currently classified as a streptobacterium, *L. casei* var. *rhamnosus*, will grow at either temperature.

The species of lactobacilli described here include those isolated most commonly in medical laboratories. Kandler and Weiss (1986), Sharpe (1981), Hammes, Weiss and Holzapfel (1991) and Hammes and Vogel (1995) offer more comprehensive descriptions and review their classification. The review by Hammes, Weiss and Holzapfel (1991) provides a comprehensive survey of the isolation, ecophysiology, identification and application of lactobacilli. Schillinger and Lücke (1987) give an account of the lactobacilli present in meat and meat products.

There is renewed interest in lactobacilli in human medicine because of the probiotic effects of some species (see p. 658).

[redacted]

Lactobacilli are found where rich carbohydrate-containing substrates are available; they live in a variety of habitats such as on mucosal membranes of humans and animal (oral cavity, intestine and vagina), in plant materials such as silage, and in foodstuffs and agricultural products, particularly milk, cheese and

fermented milk products and in fermented beverages such as wine and cider. In some of these products the multiplication of lactobacilli brings about desirable changes, in others it causes spoilage. In the body flora lactobacilli are present in moderately large numbers in the mouth, gut and vagina but seldom predominate (Salminen, Deighton and Gorbach 1993). Members of several species of lactobacilli are found at each of these sites. In general those most often present in the body flora are: in the mouth, *L. casei*, *L. fermentum*, *L. brevis* and *L. acidophilus* (Rogosa et al. 1953); in the small intestine, *L. acidophilus*, *L. fermentum*, *L. salivarius* and *L. reuteri* (Molin et al. 1993) and in the vagina, *L. acidophilus*, *L. fermentum*, *L. casei* and *L. cellobiosus* (Sharpe 1981).

19 Morphology

Lactobacilli are in general fairly large non-sporing, gram-positive rods, but they vary in length and breadth and in old cultures tend to be gram negative. A few strains are motile by peritrichous flagella. Metachromatic granules are prominent in some species, notably *L. lactis*, *L. leichmannii* and *L. bulgaricus*. Some members form long chains with cells coiled or twisted. The thermobacteria are usually large, thick and often filamentous. Among the streptobacteria, *L. casei* is a short square-ended rod, forming chains of varying length. *L. plantarum* varies in length from coccoid to short filamentous forms. Two variants of streptococci previously classified as '*Streptococcus lactis*' with a tendency to form elongated cells have been reclassified as *L. xylosus* and *L. hordniae* (Garvie, Farrow and Phillips 1981, Schleifer et al. 1985).

19.1 Isolation and selective media

All media for the isolation of lactobacilli are complex. A widely used non-selective medium, at pH 6.2–6.4, is the MRS medium of de Man, Rogosa and Sharpe (1960). For selective isolation the acetate medium (SL) of Rogosa, Mitchell and Wiseman (1951) is the medium of choice particularly when prepared in the manner described by Sharpe (1981). The presence of Tween 80 stimulates the growth of many lactobacilli and a high content of acetate at pH 5.4 is selective for them. Sharpe (1981) recommends additional media for the isolation of lactobacilli from foods and beverages because those from specialized environments may require more specific supplements.

Colonies on agar media are usually small, 1–3 mm in diameter, with entire margins. Some species form rough colonies. Rogosa and Sharpe (1959) made the general observation that colonies of streptobacteria are smooth and those of thermobacteria are rough. Some strains isolated from foodstuffs form slime.

20 Metabolism

In the homofermentative species, the streptobacteria and the thermobacteria, glucose is broken down to lactic acid almost exclusively by the Embden–Meyerhof pathway. The heterofermentative species, the betabacteria, possess the 6-phosphogluconate pathway in which the end products are carbon dioxide, acetic acid, ethanol and lactic acid (see Kandler 1982). Because carbon dioxide is soluble in water the conventional Durham tube is inapplicable and other methods, such as a shake culture in MRS agar in a tube with a plain agar overlay as a seal, are required to demonstrate carbon dioxide production. Practically all of the streptobacteria and betabacteria, but none of the thermobacteria, ferment ribose.

The lactobacilli are acidophilic and grow best in medium at about pH 6. They are aciduric and the final pH in glucose broth with some species can be as low as 3.5. For testing the fermentation of carbohydrates, Rogosa and Sharpe (1959) recommend a medium with an initial pH of 5.5–6.0. Kits in which patterns of fermentation are determined against many different carbohydrates are often employed for identification (Hammes, Weiss and Holzapfel 1991).

Most of the lactobacilli will grow in air but grow best in an atmosphere lacking oxygen but supplemented with carbon dioxide (Rogosa and Sharpe 1959). A few are strict anaerobes. The catalase test is nearly always negative and the occasional weakly positive reaction can be attributed to a pseudocatalase action because negative benzidine tests indicate the absence of a cytochrome system (Sharpe 1981).

The temperature at which growth occurs varies with the species. Thermobacteria grow best at 37–40°C; none grows at 15°C and most will grow at 45°C. The optimum for streptobacteria is about 30°C; all grow at 15°C. Among the betabacteria *L. brevis*, *L. buchneri* and *L. viridescens* resemble streptobacteria, *L. fermentum* resembles thermobacteria, *L. cellobiosus* is variable in this characteristic.

20.1 Other nutritional requirements

The nutritional requirements of lactobacilli are complex and varied but are normally met by media which, in addition to fermentable carbohydrate, contain peptone, meat and yeast extract. Supplements that are stimulatory, or even essential, include tomato juice, manganese, acetate and oleic acid esters and Tween 80 in particular. Requirements for vitamins are scattered throughout the species and vitamin-dependent strains are used for bioassays. Thiamine is necessary for the growth of nearly all the heterofermentative organisms but not those that are homofermentative (Ledesma et al. 1977). Requirements for amino acids and peptides seem to be met by a combination of cell wall bound proteinases and peptidases and mechanisms for active transport across the cell membrane (Law and Kolstad 1983).

21 Cell wall composition and antigenic structure

The chief amino acid in the peptidoglycan of most species of lactobacilli is lysine, but in some it is diaminopimelic acid and in others ornithine. All of the thermobacteria considered here have interpeptide bridges of the L-lysine–D-aspartate type. Among the streptobacteria, *L. casei* has a peptidoglycan bridge of the L-lysine–D-aspartate type and *L. plantarum* one of the *meso*-diaminopimelic type. Three types of peptidoglycan are found in the species of betabacteria

antibiotics for the treatment of these patients has been difficult (Bayer et al. 1978).

25 Role in Normal Human Flora

Lactobacilli are members of the commensal microflora of human mucosal membranes in the mouth, intestines and vagina, although they usually comprise a minor part of the flora (London 1976). In the oral cavity lactobacilli usually amount to less than 1% of the microflora although their proportion increases in individuals with a frequent intake of sugar. Studies of the intestinal lactobacillus flora of piglets have demonstrated a rapid turnover of clones (Tannock, Fuller and Pedersen 1990).

Due to production of bacteriocins and to their acidogenic potential, which reduces the pH in the local environments, lactobacilli play an important role in inhibiting the establishment of potential pathogens on mucosal surfaces (Roach and Tannock 1979, Hentjes 1983). Although the probiotic effect of lactobacilli is an old concept described by Metchnikoff in 1901 (see review by Bibel 1988), there is considerable renewed interest in this topic. Recent studies have demonstrated that lactobacilli administered orally to patients with viral and bacterial intestinal infections augment mucosal immune responses and promote recovery (Kaila et al. 1992, Perdigon et al. 1995). Furthermore, it has been demonstrated in a rat model that lactobacilli increase the barrier functions of the gut mucosa (Isolauri et al. 1993).

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described here: (1) L-lysine–D-aspartate in *L. brevis* and *L. buchneri*; (2) L-ornithine–D-aspartate in *L. fermentum* and *L. cellobiosus*; and (3) L-lysine–L-alanine–L-serine in *L. viridescens* (Schleifer and Kandler 1972).

Many lactobacilli share an antigen present in the cell membrane (Sharpe et al. 1973a). This corresponds to the 1–3 linked glycerol phosphate units of the membrane glycerol teichoic acid. This antigen is distinct from the group antigens described by Sharpe (1955), which she identified by precipitation reactions between hot-acid extracts and antisera. The chemical composition of these antigens was reviewed by Knox and Wicken (1976) and is shown in Table 28.4. It will be noted that the *L. casei* strains, except those of subspecies *rhamnosus*, may possess one of 2 group antigens, and that the group E antigen occurs in members of 4 species, 2 of them thermobacteria and 2 betabacteria.

Susceptibility to physical and chemical agents

The lactobacilli have no particular resistance to heat and are destroyed by exposure to 60° or 65° for 30 min. They are, however, specially resistant to acid and are able to grow in concentrations of acid that are fatal to most other bacteria. The tolerance to bile varies and has been used to distinguish between species (Sharpe 1981).

Lactobacilli of several species can be resistant to many antibiotics including vancomycin, the peptide antibiotics, the macrolides, tetracycline and the aminoglycosides. In these strains loss of plasmids was usually accompanied by a change to sensitivity with several antibiotics (Vescovo, Morelli and Bottazzi 1982).

Lactobacilli are refractory to transformation and transduction, but the transmission of the plasmid that determines the ability of *L. casei* to ferment lactose does occur naturally.

Classification

Orla-Jensen (1919, 1943), whose monographs have influenced all subsequent workers, laid particular stress on fermentative ability and the type of lactic acid produced from glucose in his classification of lactobacilli. His 3 primary divisions of the lactobacilli into thermobacteria, streptobacteria and betabacteria were followed for many years, though not his proposal to consider them as separate genera. However, recent comparative studies of 16S rRNA sequences revealed that the 3 groups lack phylogenetic foundation. Furthermore, such studies indicate that lactobacilli are phylogenetically intermixed with members of the genera *Leuconostoc* and *Pediococcus* despite differences in morphology and fermentation patterns (for review see Schleifer and Ludwig 1995). Three phylogenetic groups are evident although the first 2 may be difficult to distinguish:

1 *Obligate homofermenters*. This group includes the type species *L. delbrueckii* and other obligately homofermentative lactobacilli including *L. acidophilus*.
2 *Facultative heterofermenters*. This group comprises more than 80 *Lactobacillus* species including *L. rhamnosus*, *L. intestinalis*, and *L. sake* most of which are facultatively heterofermentative. In addition to the lactobacilli the group includes 5 *Pediococcus* species.
3 *Obligate heterofermenters* which are closely related to the leuconostocs.

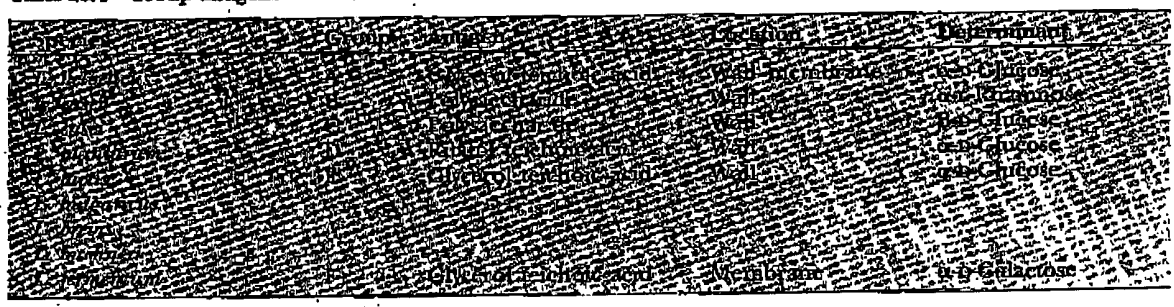
These sequence studies also revealed that several taxa were erroneously placed in the genus *Lactobacillus*. As a result some were transferred to the genus *Clostridium* and 2 recently described anaerobic species '*L. ulf*' and '*L. rimae*' isolated from human gingival crevices (Olsen et al. 1991) were transferred to the new genus *Atopobium* (Collins and Wallbanks 1992).

Hammes and Vogel (1995) have proposed a grouping based on a combination of phylogenetic data and biochemical and physiological characteristics of the species. They provide details about cell wall composition and identification criteria. It is remarkable that for an unequivocal identification of a *Lactobacillus* isolate it is not always sufficient to use the classical physiological and biochemical tests. A review by Pot et al. (1994) provides a discussion of methods for identification of lactobacilli including a critical evaluation of their limitations.

Pathogenicity

The relationship of lactobacilli to dental caries has been reviewed (Hardie and Bowden 1974). Lactobacilli are occasionally isolated from bacteraemic patients (Sharpe, Hill and Lapage 1973b). The selection of

Table 28.4 Group antigens of lactobacilli



Based on Knox and Wicken (1976).